Application No. 10/031,331

Applicants: Akioy YAMADA et al.

The listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:** 

1. (Original) A method for screening DNA encoding proteins having the activity of

improving environmental stress tolerance wherein candidate cDNA derived from cDNA library

is introduced into host cells, the obtained transformed cells are cultured under the conditions

where the host cells cannot substantially grow, the clones grown after culturing are selected, and

the candidate cDNA introduced from the selected clones is isolated.

2. (Original) A method for screening DNA encoding proteins having the activity of

improving environmental stress tolerance wherein candidate cDNA derived from cDNA library

is introduced into host cells, the obtained transformed cells are cultured under conditions where

the host cells cannot substantially grow, the clones grown after the culturing are selected, the

candidate cDNA introduced from the selected clones is isolated, the isolated candidate cDNA is

introduced into the isolated cDNA, the mutant cDNA is introduced into host cells, and the

process of selecting is repeated one or more under stringent conditions of selecting mutant cDNA

than the selecting condition.

3. (Previously Presented) The method for screening according to claim 1, wherein the

environmental stress is one or more of chemical substance stress, high temperature stress, low

temperature stress, freezing stress, drought stress, ozone stress, ultraviolet stress, radiation stress,

or osmotic pressure stress.

4. (Original) The method for screening according to claim 3, wherein the chemical

substance stress is salt stress.

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- 5. (Previously Presented) The method for screening according to claim 1, wherein the host cell is a coliform.
- 6. (Original) The method for screening according to claim 5, wherein the coliform is SOLR strain.
- 7. (Previously Presented) The method for screening according to claim 1, wherein an environmental condition where host cells cannot substantially grow is 350mM or more of salt concentration.

## 8-12. (Canceled)

- 13. (Original) DNA encoding proteins according to any one of the following (a) to (c):
  - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 2,
- (b) a protein comprising a sequence of amino acids having 70% or more of homology with the sequence of amino acids shown in Seq. ID No. 2, and having the activity of tolerance at least against salt stress,
- (c) a protein having a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 2, and having the activity of improving tolerance at least against salt stress.
- 14. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 1, or its complementary sequence.

- 15. (Original) DNA hybridized with the DNA according to claim 14 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress.
  - 16. (Original) DNA encoding proteins according to any one of the following (a) or (b):
    - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 4,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 4, and having the activity of improving tolerance at least against salt stress.
- 17. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 3 or its complementary sequence.
- 18. (Original) DNA hybridized with the DNA according to claim 17 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress.
  - 19. (Original) DNA encoding proteins according to any one of the following (a) or (b):
    - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 6,
- (b) a protein comprising the sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 6, and having the activity of improving tolerance at least against salt stress.

- 20. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 5 or its complementary sequence.
- 21. (Original) DNA hybridized with the DNA according to claim 20 under stringent conditions, and encoding proteins comprising the activity of improving tolerance at least against salt stress.
  - 22. (Original) DNA encoding proteins according to any one of the following (a) or (b):
    - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 8,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 8, and having the activity of improving tolerance at least against salt stress.
- 23. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 7 or its complementary sequence.
- 24. (Original) DNA hybridized with the DNA according to Claim 23 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress.
  - 25. (Original) DNA encoding proteins according to any one of the following (a) or (b):
    - (a) a protein comprising a sequence of amino acids shown in Seq. ID No. 10,

(b) a protein comprising a sequence of amino acids wherein one or more of amino

acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 10,

and having the activity of improving tolerance at least against salt stress.

26. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID

No. 9 or its complementary sequence.

27. (Original) DNA hybridized with the DNA according to claim 26 under stringent

conditions, and encoding proteins having the activity of improving tolerance at least against salt

stress.

28. (Original) DNA encoding proteins according to any one of the following (a) or (b):

(a) a protein having the sequence of amino acids shown in Seq. ID No. 12,

(b) a protein having a sequence of amino acids wherein one or more of amino acids

are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 12, and

having the activity of improving tolerance at least against salt stress.

29. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID

No. 11 or its complementary sequence.

30. (Original) DNA hybridized with the DNA according to claim 29 under stringent

conditions, and encoding proteins having the activity of improving tolerance at least against salt

stress.

31. (Original) DNA encoding proteins according to any one of the following (a) or (b):

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- (a) a protein having the sequence of amino acids shown in Seq. ID No. 14,
- (b) a protein having a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 14, and having the activity of improving tolerance at least against salt stress.
- 32. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 13 or its complementary sequence.
- 33. (Original) DNA hybridized with the DNA according to claim 32 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress.
  - 34. (Original) DNA encoding proteins according to any one of the following (a) or (b):
    - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 16,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 16, and having the activity of improving tolerance at least against salt stress.
- 35. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 15 or its complementary sequence.
- 36. (Original) DNA hybridized with the DNA according to claim 35 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress.

- 37. (Original) DNA encoding proteins according to any one of the following (a) or (b):
  - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 18,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 18, and having the activity of improving tolerance at least against salt stress.
- 38. (Original) DNA having part or whole of the sequence of bases shown in Seq. ID No. 17 or its complementary sequence.
- 39. (Original) DNA hybridized with the DNA according to Claim 38 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress.
  - 40. (Original) DNA encoding proteins according to any one of the following (a) or (b):
    - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 20,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 20, and having the activity of improving tolerance at least against salt stress.
- 41. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 19 or its complementary sequence.

- 42. (Original) DNA hybridized with the DNA according to claim 41 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress.
  - 43. (Original) DNA encoding proteins according to any one of the following (a) or (b):
    - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 22,
- (b) a proteins comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 22, and having the activity of improving tolerance at least against salt stress.
- 44. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 21 or its complementary sequence.
- 45. (Original) DNA hybridized with the DNA under stringent conditions according to claim 44, and encoding proteins having the activity of improving tolerance at least against salt stress.
  - 46. (Original) DNA encoding proteins according to any one of the following (a) or (b):
    - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 24,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 24, and having activity of improving tolerance at least against salt stress.

- 47. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 23 or its complementary sequence.
- 48. (Original) DNA hybridized with the DNA according to Claim 47 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress.
  - 49. (Original) DNA encoding proteins according to any one of the following (a) or (b):
    - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 26,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 26, and having the activity of improving tolerance at least against salt stress.
- 50. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 25 or its complementary sequence.
- 51. (Original) DNA hybridized with the DNA according to Claim 50 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress.
  - 52. (Original) DNA encoding proteins according to any one of the following (a) or (b):
    - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 28,

(b) a protein comprising a sequence of amino acids wherein one or more of amino

acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 28,

and having the activity of improving tolerance at least against salt stress.

53. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID

No. 27 or its complementary sequence.

54. (Original) DNA hybridized with the DNA according to claim 53 under stringent

conditions, and encoding proteins having the activity of improving tolerance at least against salt

stress.

55. (Original) DNA encoding proteins according to any one of the following (a) or (b):

(a) a protein comprising the sequence of amino acids shown in Seq. ID No. 30,

(b) a protein comprising a sequence of amino acids wherein one or more of amino

acids are deleted, substituted, or added in the sequence of amino acids shown in. ID No. 30, and

having the activity of improving tolerance at least against salt stress.

56. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID

No. 29 or its complementary sequence.

57. (Original) DNA hybridized with the DNA according to Claim 56 under stringent

conditions, and encoding proteins having the activity of improving tolerance at least against salt

stress.

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- 58. (Original) DNA encoding proteins according to any one of the following (a) or (b):
  - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 32,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 32, and having the activity of improving tolerance at least against salt stress.
- 59. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 31 or its complementary sequence.
- 60. (Original) DNA hybridized with the DNA according to claim 59 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress.
  - 61. (Original) DNA encoding proteins according to any one of the following (a) or (b):
    - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 34,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 34, and having the activity of improving tolerance at least against salt stress.
- 62. (Original) DNA comprising part or whole of the sequence of bases shown in Seq. ID No. 33 or its complementary sequence.

- 63. (Original) DNA hybridized with the DNA according to Claim 62 under stringent conditions, and encoding proteins having the activity of improving tolerance at least against salt stress.
- 64. (Currently amended) An isolated DNA encoding a protein according to any one of the following (a) or (b):
  - (a) a protein comprising the sequence of amino acids shown in Seq. ID No. 40,
- (b) a protein comprising a sequence of amino acids wherein one or more of amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 40, and having the activity of improving tolerance at least against salt stress.
- 65. (Currently Amended) An isolated DNA comprising part or all of the sequence of bases shown in Seq. ID No. 39, or its complementary sequence.
- 66. (Currently Amended) An isolated DNA which hybridizes with the DNA according to claim 65 under stringent conditions for hybridization at 42°C and washing treatment using a washing buffer containing 1XSSC, 0.1% SDS at 42°C, and encodes proteins a protein having the activity of improving tolerance at least against salt stress.

67-69. (Canceled)

70. (Original) A protein comprising of the sequence of amino acids shown in Seq. ID No. 2.

- 71. (Original) A protein having 70% or more of homology with the sequence of amino acids shown in Seq. ID No. 2, and having the activity of improving tolerance at least against salt stress.
- 72. (Original) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 2, and having the activity of improving tolerance at least against salt stress.
- 73. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 4.
- 74. (Original) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 4, and having the activity of improving tolerance at least against salt stress.
- 75. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 6.
- 76. (Original) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq.-ID--No. 6, and having the activity of improving tolerance at least against salt stress.
- 77. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 8.

78. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 8, and having the activity of improving tolerance at least against salt stress.

79. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No.

10.

80. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 10, and having the activity of improving tolerance at least against salt stress.

81. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No.

12.

82. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 12, and having the activity of improving tolerance at least against salt stress.

83. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No.

14.

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84. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 14, and having the activity of improving tolerance at least against salt stress.

85. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No.

16.

86. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 16, and having the activity of improving tolerance at least against salt stress.

87. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No.

18.

88. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 18, and having the activity of improving tolerance at least against salt stress.

89. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No.

20.

90. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 20, and having the activity of improving tolerance at least against salt stress.

- 91. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 22.
- 92. (Original) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 22, and having the activity of improving tolerance at least against salt stress.
- 93. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 24.
- 94. (Original) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 24, and having the activity of improving tolerance at least against salt stress.
- 95. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 26.
- 96. (Original) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 26, and having the activity of improving tolerance at least against salt stress.
- 97. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No. 28.

98. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 28, and having the activity of improving tolerance at least against salt stress.

99. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No.

30.

100. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 30, and having the activity of improving tolerance at least against salt stress.

101. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No.

32.

102. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 32, and having the activity of improving tolerance at least against salt stress.

103. (Original) A protein comprising the sequence of amino acids shown in Seq. ID No.

34.

104. (Original) A protein comprising a sequence of amino acids wherein one or more

amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID

No. 34, and having the activity of improving tolerance at least against salt stress.

105. (Previously presented) A protein comprising the sequence of amino acids shown in Seq. ID No. 40.

106. (Previously presented) A protein comprising a sequence of amino acids wherein one or more amino acids are deleted, substituted, or added in the sequence of amino acids shown in Seq. ID No. 40, and having the activity of improving tolerance at least against salt stress.

107. (Previously Presented) An antibody specifically bound to the protein according to claim 70.

108. (Previously Presented) An antibody specifically bound to the protein according to claim 73.

109. (Previously Presented) An antibody specifically bound to the protein according to claim 105.

110. (Previously Presented) The antibody according to claim 107, wherein the antibody is a monoclonal antibody.

## 111-113. (Canceled)

114. (Previously presented) A vector comprising the DNA according to any one of claims 64-66.

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115. (Previously Presented) A transformed cell obtained by introducing the vector

according to claim 114 to a host cell.

116. (Original) A transformed cell according to claim 115, wherein the host cell is a

plant cell.

117. (Previously Presented) A method for producing a protein having the activity of

improving environmental stress tolerance, wherein the transformed cells according to claim 115

is cultured, and a recombinant protein is collected from the transformed cells or the supernatant

of the cultured liquid.

118. (Canceled)

119. (Previously Presented) A transgenic plant obtained by introducing the DNA

according to claim 13 encoding proteins having the activity of improving environmental stress

tolerance, and by dividing, proliferating and redifferentiating the plant cell.

120. (Previously Presented) A transgenic plant obtained by introducing the DNA

according to claim 16 encoding proteins having the activity of improving environmental stress

tolerance, and by dividing, proliferating and redifferentiating the plant cell.

121. (Currently Amended) A transgenic plant obtained by introducing the DNA

according to any one of claims 64 to 66 to a plant cell and regenerating a plant from the plant cell

by dividing, proliferating and redifferentiating the plant cell.

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122. (Currently Amended) A transgenic plant obtained by introducing the vector according to claim 114 to a plant cell, and regenerating a plant from the plant cell by dividing, proliferating and redifferentiating the plant cell.

123-124. (Canceled)

125. (Currently Amended) A material for breeding transgenic plant part which is derived from the transgenic plant according to claim 121.